From Page No. 86

Spun down pSV15/FUS 29 & pSV15/HPTK6 (Full leng
washed 3x 1ml -20°C 70% EtOH
Dried
R.S'd each in 400µl TE
Measured O.D$_{280}$ of 10µl each (in 1ml)

HPTK6
(Full length) = 0.382 x 50 = 19.1 µg/ml ÷ 10 = 1.91

Fus 29 = 0.416 x 50 = 20.8 ÷ 10 = 2.08 µg/ml

Picked up DP 12 (CHO) cells for transfection on 5/14

Have 50ml put into a spinner flask

Added 100ml
F12 + 5% dFBS
+ 10mM HEPES
+ 1x GHT

Inc 37°C O/N.

Genentech, Inc.
Genentech, Inc.
Genentech, Inc.
Genentech, Inc.
Genentech, Inc.

INTERNAL TRANSMITTAL FORM

TO Will Baron	[redacted]	DEPARTMENT NAME AND COST CENTER NO. Mol. Bio	409
FROM C-Nigel	C-nagel [redacted]	DEPARTMENT NAME AND COST CENTER NO. CBL	613
PROJECT NAME DP-12	700-215	EXPIRATION DATE/TIME	AMOUNT 50ml
POINT CODE NO.		LOT NO. OR NOTEBOOK NO. AND PAGE 10599-	
STORAGE CONDITIONS			
SPECIAL INSTRUCTIONS d27/p23 BSA +5% NFBS +1xGHT count 22.8x10^5 C/ml (98%)			

RECIPIENT

To Page No. 87

Witnessed & Understood by me,	Date	Invented by Will Baron	Date [redacted]
		Recorded by	

From Page No. 87

RD's for transfection

(A) full length

26.2µl DNA (50µg)
10µl 10x H
61.8µl H_2O
2µl Not I (80U)
100

(B) Fusion

24µl DNA (50µg)
10µl 10x H
64µl H_2O
2µl Not I (80U)
100

Inc both 37°C 3hrs

Saved 1µl each for gel analysis

Extracted each 1x 100µl 50/50 phenol/CHCl3
1x 100µl CHCl3

Added 10µl 3M NaAc pH 4.8 to each
Added 220µl RT 100% EtOH to each

Mixed & spun full speed, RT 5'

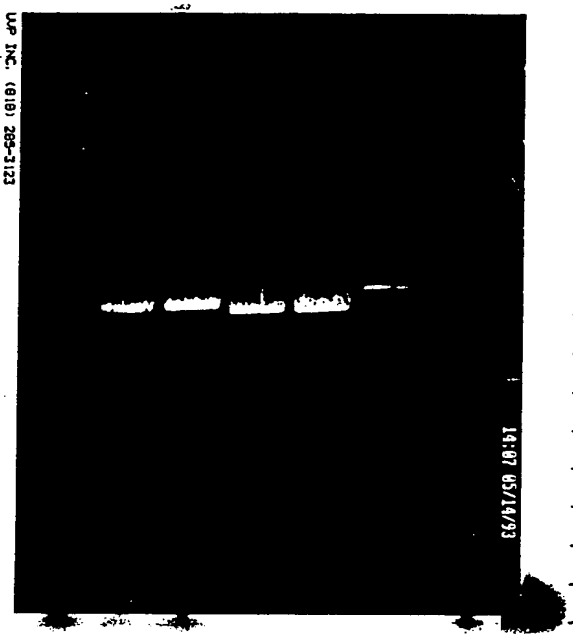
Decanted
Washed pellets 2x 1ml -20°C 70% EtOH
Respun → decanted
RS'd each in 100µl H_2O → kept on ice
Removed 1µl each for gel analysis
Ran pre & post ppt samples on 0.7% agarose (1x TBE)

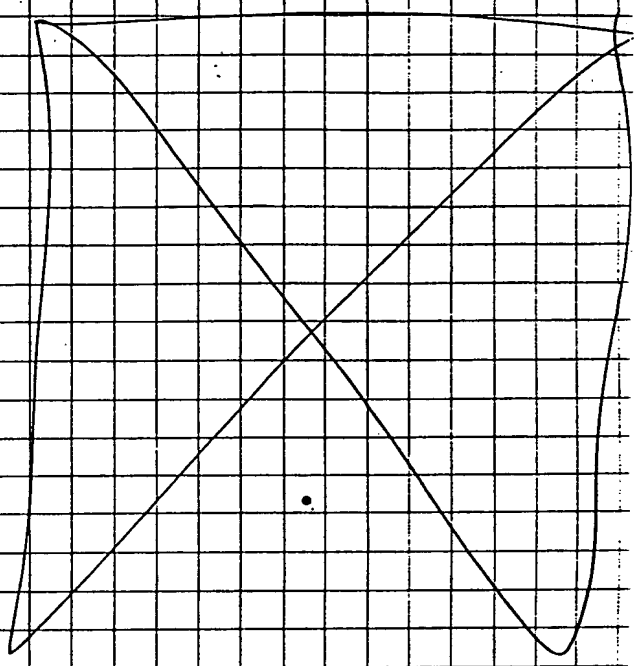
To Page No. 89

Witnessed & Understood by me, | Date | Invented by | Recorded by WMBaron | Da[redacted]

From Page No. 88

READY!





Counted 200µl of DP12 cells in coulter counter
Have 7.6×10^5 cells/ml
need 1×10^7 cells/transfection = 13.2ml
Spun down 2x 13.2ml ~2K RPM GPR 4°C 5'
Decanted & RS'd each in 700µl 2x Hepes buffered saline
Placed in pre-chilled Bio-Rad 0.4cm electrocuvette
Added DNA's to cells
Electroporated 2.5µF, 0.5Kvolts
Got 0.4 ms (time constant) → Removed from cuvette

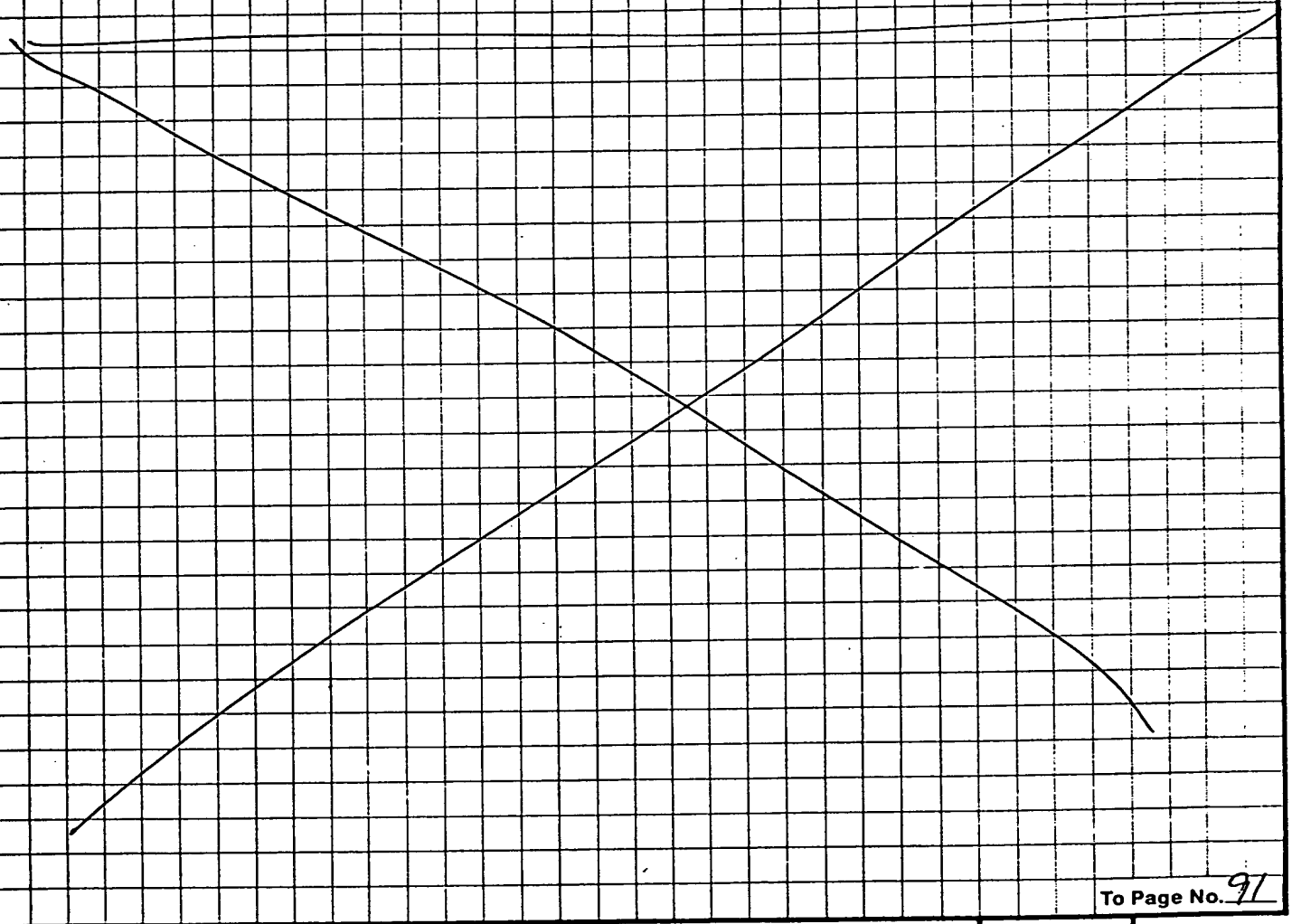
To Page No. 91

Witnessed & Understood by me,	Date	Invented by	Date
		Recorded by UM Bacon	[redacted]

90 Project No. 713 Book No. 17522 TITLE

From Page No. 89

Plated each transfection onto 4×100mm tissue culture plates w/ 10ml F12 DMEM 5% dFBS 10mM HEPES 1× G418 Inc @ 37°C w/ 5% CO_2 O/N.



To Page No. 91

Witnessed & Understood by me, Date

Invented by Will Bacon

Recorded by [redacted]

Date [redacted]

From Page No. 90

Checked cells → I don't have a proper microscope, but it appears that they mostly all stuck down.

Changed media (after 24 hrs incubation) to F12 + 5% dFBS
DMEM + 10 mM HEPES.

(+) GHT (for selection of DHFR intron on pSV15)

Note: I may have accidentally used 100 mM HEPES on 5/14 after electroporation. I will have to wait & see if this causes a problem.

To Page No. 92

Witnessed & Understood by me, | Date | Invented by W.W. Bacon | Recorded by | Date [redacted]

Project No. 713

92 Book No. 17522 TITLE

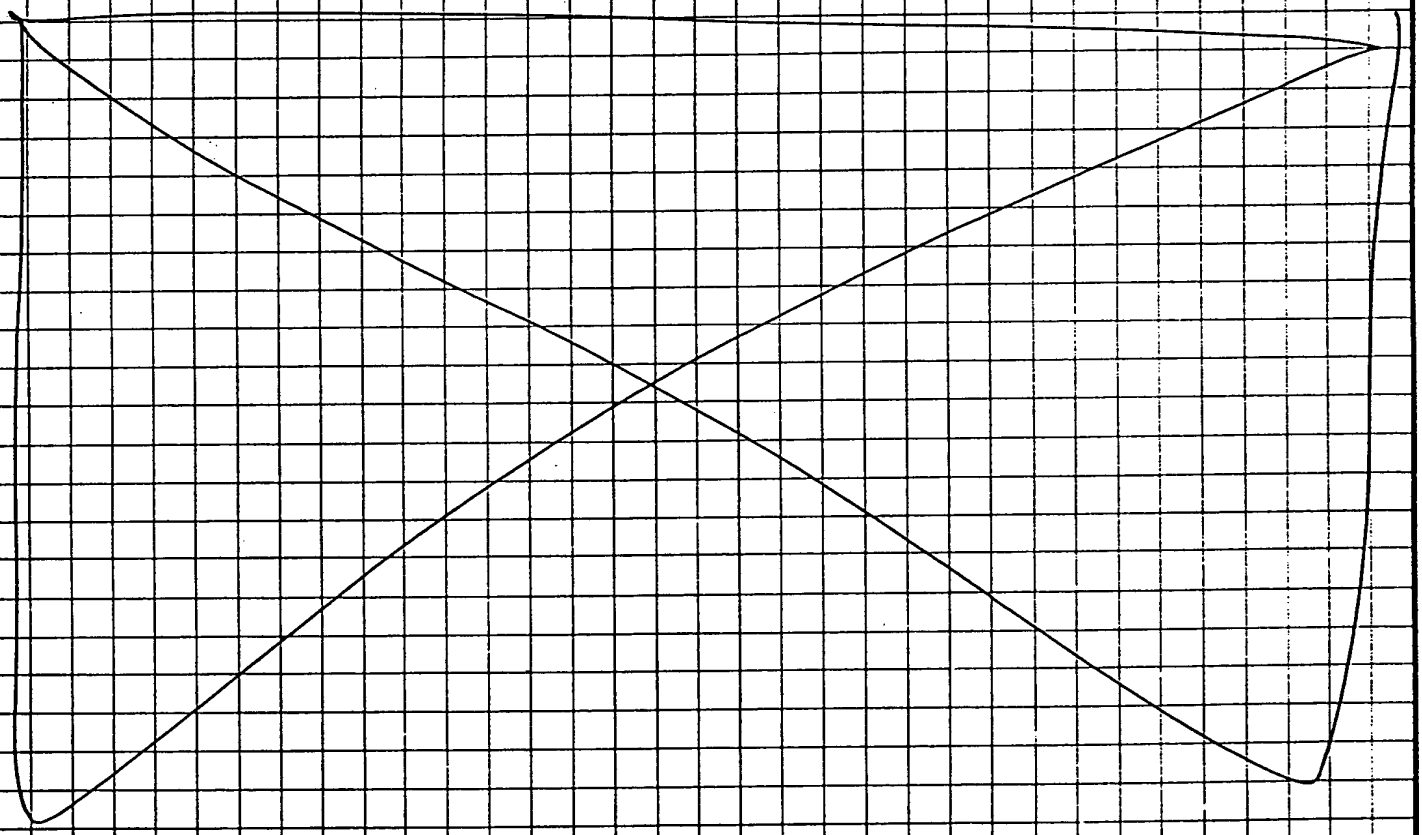
From Page No. 91

Got a tissue culture scope today + checked cells → good deal

Many dead ones in there, but still plates are ~ 50% confluent.

Changed media

Continued Inc 37°C o/n 5% CO_2.



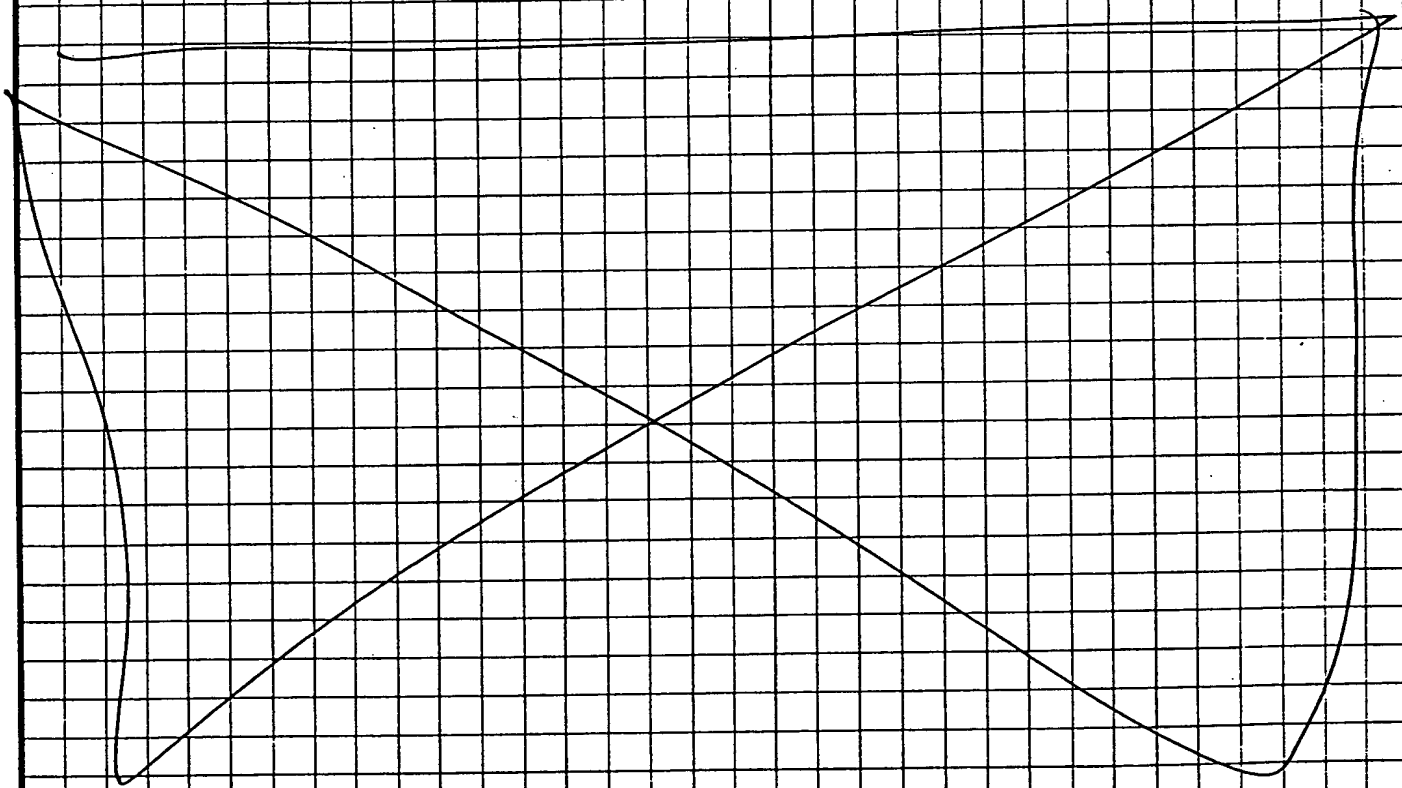
To Page No. 93

Witnessed & Understood by me,	Date	Invented by W.M. Bowen	Date [redacted]
		Recorded by [redacted]	

TITLE

From Page No. 92

Checked CHO cells today. I am starting to get very much more cell death (probably due to the 6HT ⊖ selection).
I should eventually have essentially clear plates except for the isolated colonies of transformed cells.
Continued incubation @ 37°C w/ 5% CO_2.



To Page No. 94

93

Witnessed & Understood by me,

Date

Invented by

Recorded by WM Bacon

Date [redacted]

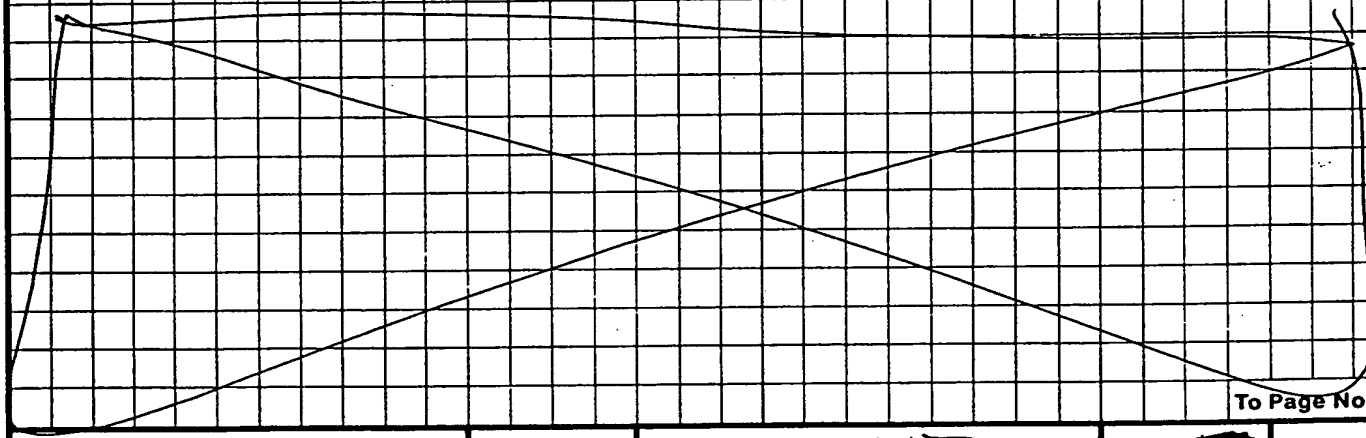
Project No. 1713
94 Book No. 17522 TITLE

From Page No. 93

Checked CHO cell cultures
HPTK6 full length
HPTK6 / Ig6 Fusion } both in pSV15.ED.LL

Major cell death is occurring
~ 90% of cells are dead.
Micro-colonies are starting to appear → each containing ~ 10-20 cells as of now.
Changed media on all plates with 10 ml fresh 37°C pre-warmed
F12 + 10mM HEPES
DMEM + 5% dFBS

Continued incubation 37°C O/N w/ 5% CO_2.



To Page No. 95

Witnessed & Understood by me, | Date | Invented by | Date
Recorded by

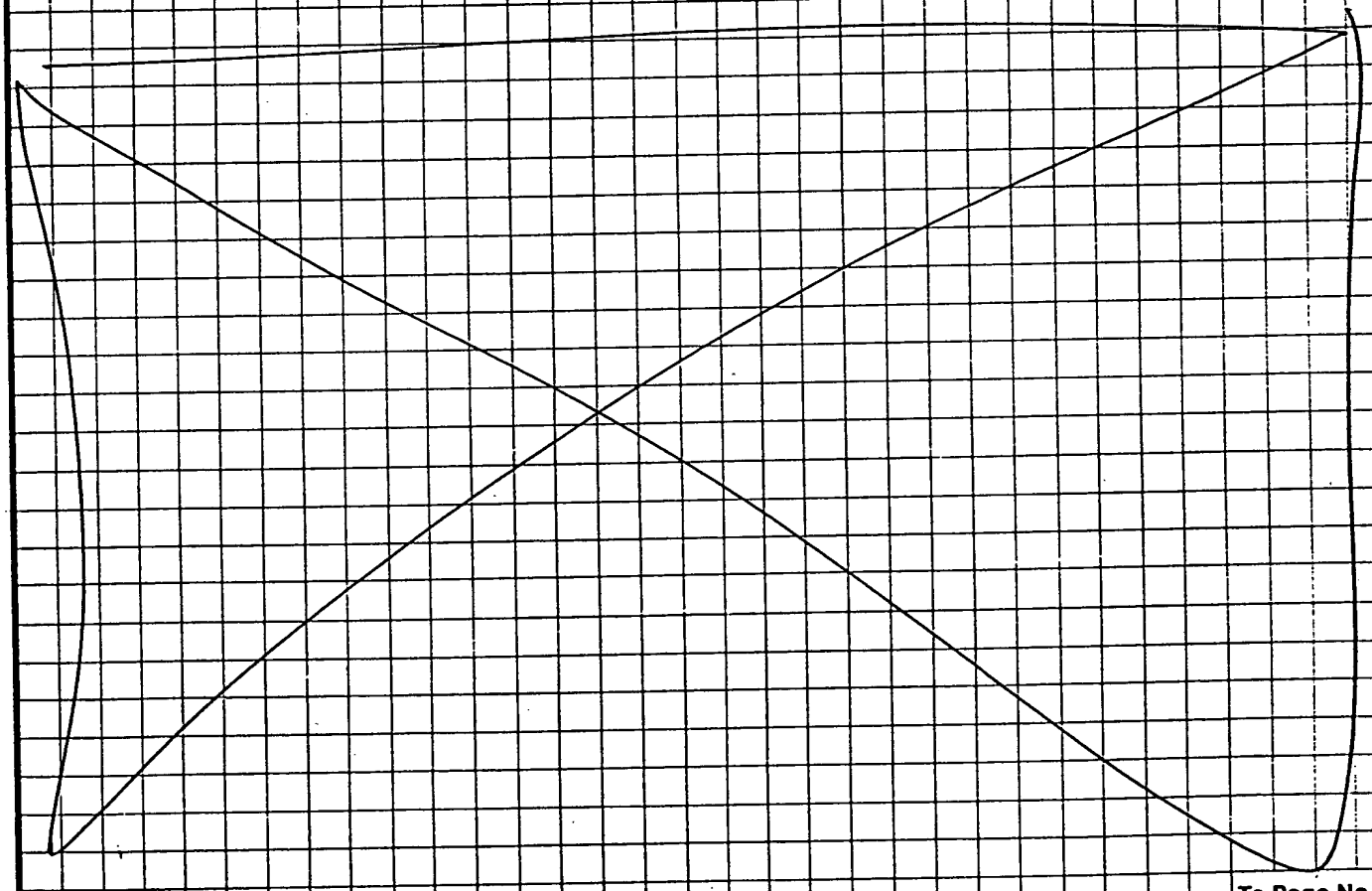
Proj. No. 1713
Book No. 17522

TITLE

From Page No. 94

Checked CHO cell transfections.
Cell death is continuing
Small colonies are still apparent
(I would guesstimate ~ 20/plate
and maybe a few more).

Cont Inc 37°C 5% CO_2



To Page No. 96

Witnessed & Understood by me,	Date	Invented by	Date [redacted]
		Recorded by W.M. Bacon	

Project No. 1713

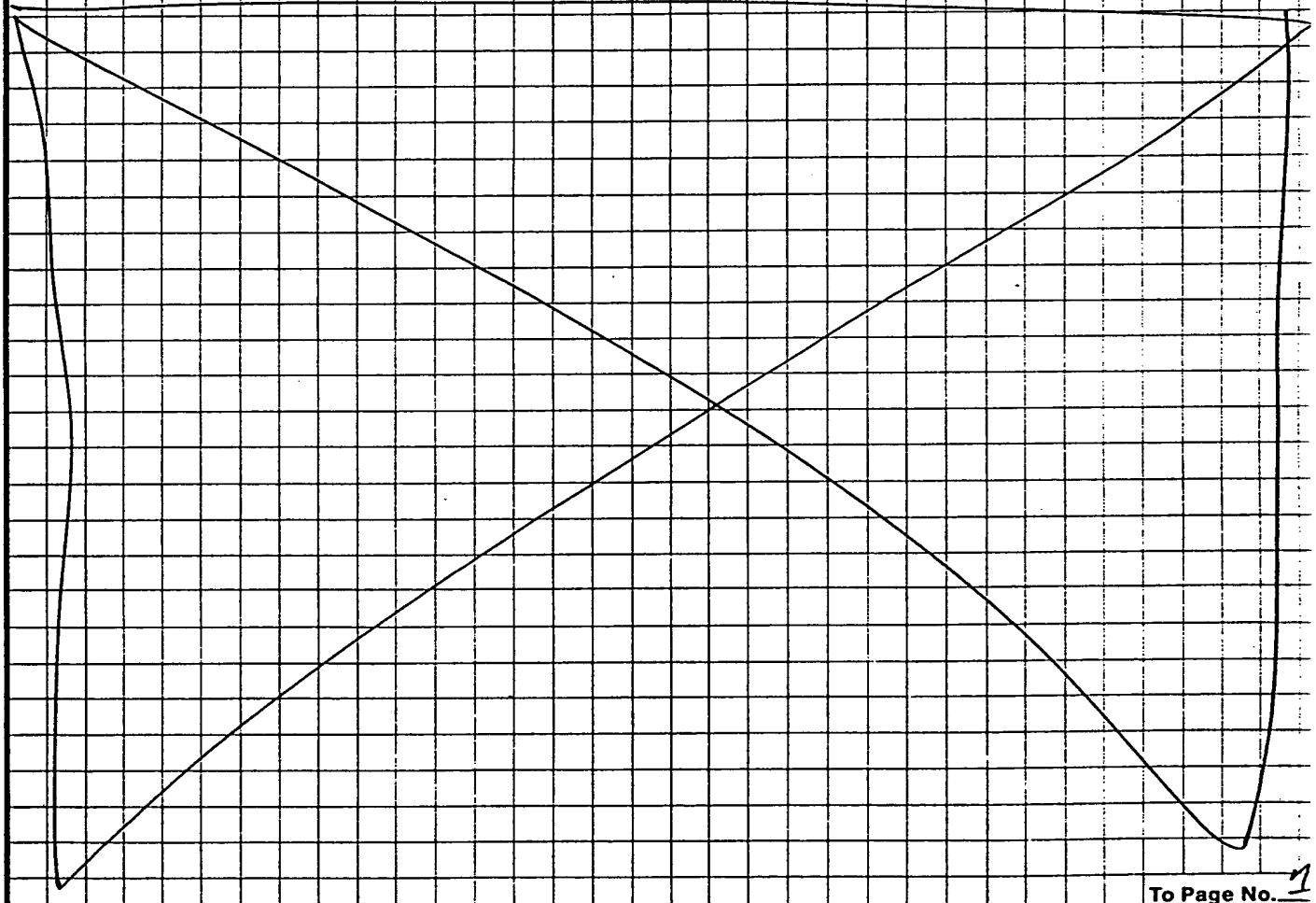
9 96 Book No. 17522 TITLE

From Page No. 95

Changed media on HPTK6 & HPTK6/FL5 transfection plates

Replaced each w/ 10 ml F12 DMEM + 10mM HEPES 5% d FBS

Cont @ 37°C, 5% CO_2 until 5/24/93



To Page No. 1

Witnessed & Understood by me,	Date	Invented by	Date [redacted]	Book # 18002
		Recorded by		

TITLE

Project No

Book No

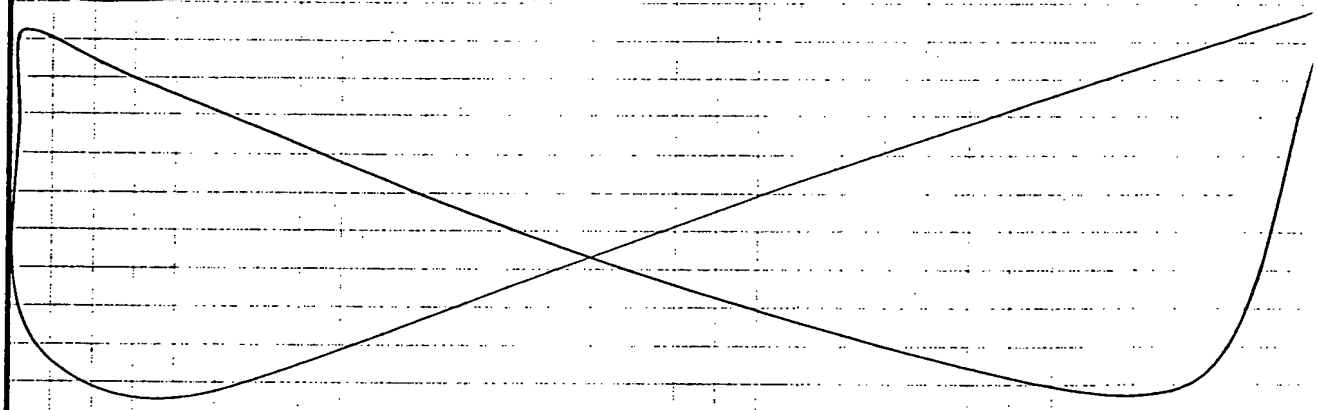
Page

From Page No. 96 Book # 17522

Checked CHO cells (HPTK6/IgG1 FUS + HPTK6 F
cells look fine → colonies are looking goo
but are still too small to be seen
by naked eye. Colonies may be
pickable by next week.
Changed media on all plates
Put 10ml fresh onto each one.
Media is

F12: DMEM
5% dFBS
10mM HEPES.

Cont. Inc @ 37°C w/ 5% CO2.



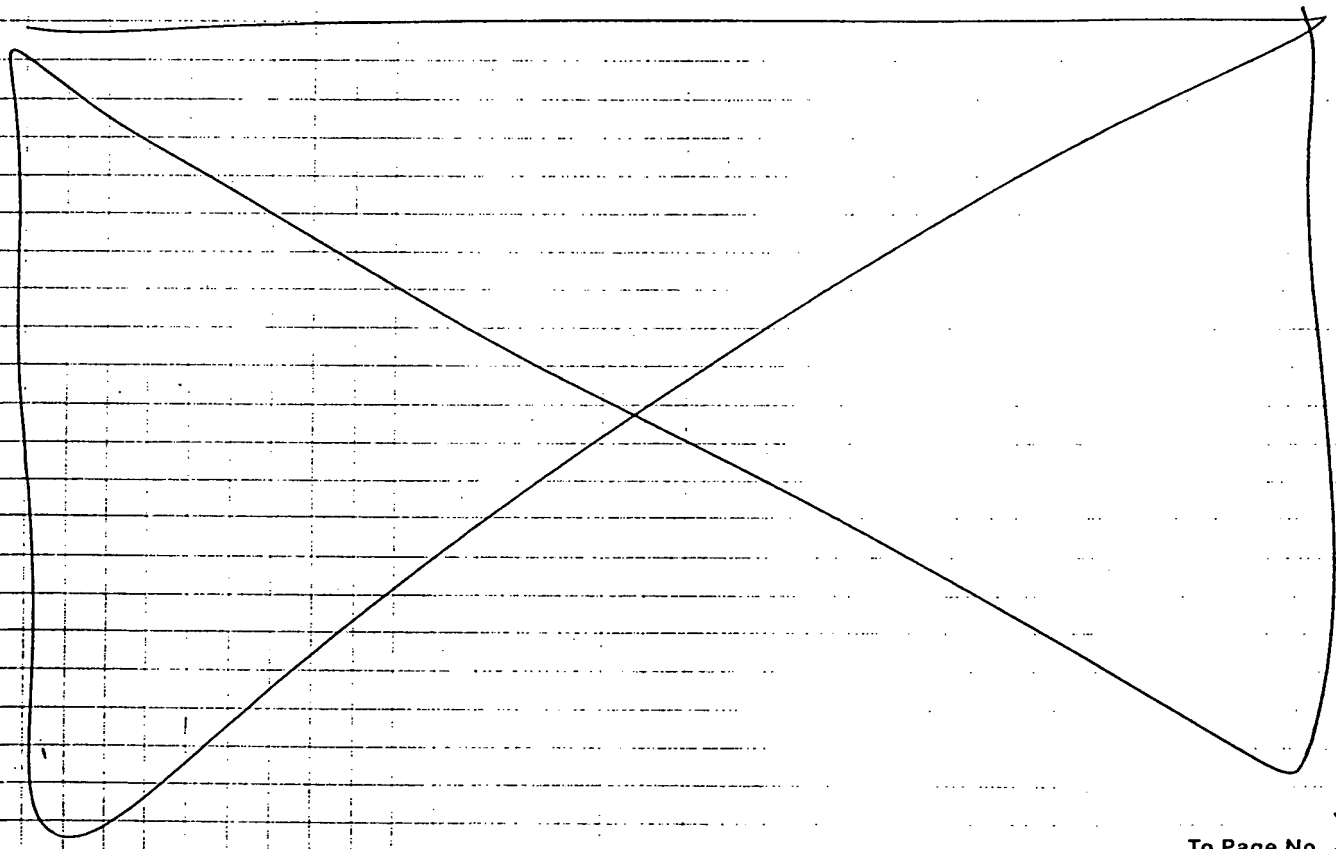
To Page

Witnessed & Understood by me,	Date	Invented by	Dat[redacted]
		Recorded by WM Bacon	

2 Project No. 1713
Book No. 18002 TITLE

From Page No. 7

Checked CHO cell transfection plates.
No contamination
Cells look healthy
Colonies are increasing in size.
Plate is being "seeded" from larger colonies.
Cont. Inc @ 37°C w/ 5% CO_2.



To Page No. 3

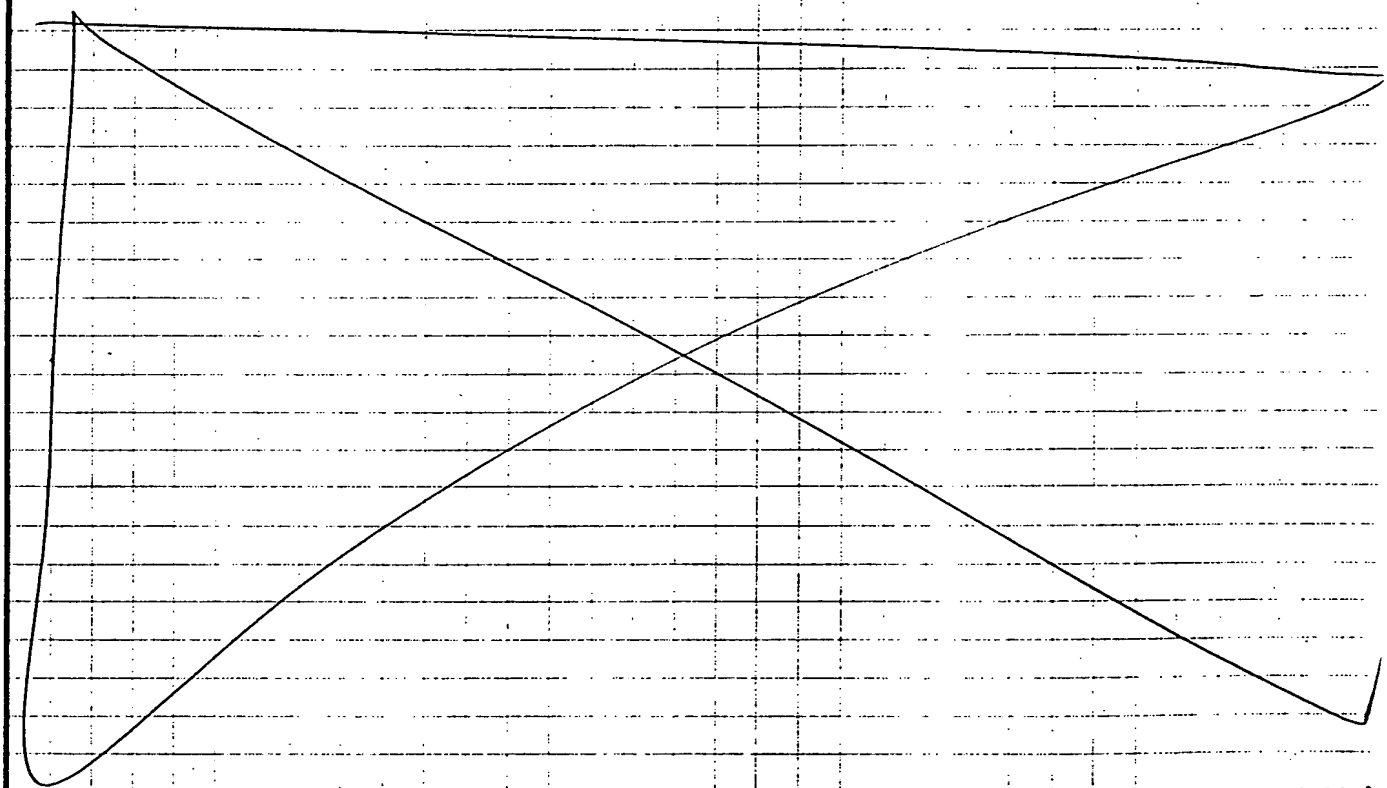
Witnessed & Understood by me,	Date	Invented by	Da[redacted]
		Recorded by CMM Bacon	[redacted]

From Page No. 2

Checked cells → still looking fine
Colonies are now visible to naked eye
but are still way too small to pick.
Changed media → put 10ml fresh on each pl

F12: DMEM
5% of FBS
10 mM HEPES

Cont Incu @ 37°C 5% CO2.



To Page No. 4

Witnessed & Understood by me,	Date	Invented by	Date
		Recorded by Will Bowen	[redacted]